MITOSIS IN AMŒBA PROTEUS

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In 1935 the authors described the process of mitosis in *Amœba dubia.* This process was found to be so different from that described by other workers on large free-living amœbæ that it was considered desirable to investigate the phenomena attendant on fission in *Amœba proteus,* the other most common large free-living amœba. The first mitotic figure, a metaphase, to be described for *Amœba proteus* was that of Carter (1912). This author succeeded in obtaining and figuring this single stage of the process. In 1918, Doflein described a few mitotic stages. His description was incomplete for early and final stages of the process. Both Doflein and Levy (1924) figure and describe the division sphere of *Amœba proteus.* Levy's figures are in outline only and he includes no nuclear stages. Taylor (1923), evidently working with *Amœba proteus,* described phenomena which may be reasonably interpreted as pathological. Bělař (1926) describes from original but unpublished work several stages in mitosis. Although he considers these stages to be of mitosis in *Amœba proteus* we believe them to be of *Amœba dubia* or some closely related species. Chalkley and Daniel (1933) presented an account of mitosis in *Amœba proteus* and correlated very closely cytoplasmic configuration of division spheres with nuclear stages. These authors used only one method of fixation and one stain and all figures and descriptions were made solely from living animals or total mounts. In a later paper, Chalkley (1936) used additional stains including the Feulgen reaction. Our experience in working on mitosis in *Amœba dubia* has convinced us that a clearer understanding of the method of division in amœbæ can be had by supplementing such studies with the use of sectioned material. With this in view the present study of mitosis in *Amœba proteus* was begun.

Material and Methods

Two different strains of *Amœba proteus* Pallas (Leidy) (see Schaeffer, 1916) were used in this work. One strain was isolated from a culture collected from a pond in the vicinity of Woods Hole, Mass., in 1924 by the senior author. The second strain was obtained through

the kindness of Mr. J. I. Phinney who originally collected the amœbæ from Jamaica Pond, Boston, Mass. Specimens of the latter have been cultured in this laboratory since March, 1936. The method of culture of both is essentially similar to that published by Dawson (1928).

The technique for isolating, fixing, staining, etc. was similar to that used in the work on *Amœba dubia* (1935).

Dividing amœbæ were obtained in profusion at any desired time by the following method. Thriving cultures were placed in a refrigerator at a temperature of 3° to 4° C. overnight and set out next morning at room temperature, approximately 27° to 29° C. Depending upon the room temperature, amœbæ were found dividing in the greatest number at periods varying from 2 to 4 hours after removal from the refrigerator. This method obviates much tiresome watching of cultures for dividing amœbæ.

The following fixatives were used: Schaudinn's, Schaudinn's with 5 per cent glacial acetic, Bouin's (Allen's B–15 modification), Flemming weak and Carnoy and Lebrun's fluid. For total mounts both Carnoy-Lebrun and weak Flemming were used. In sectioned material for finer detail Flemming proved best.

The following stains were used: Heidenhain's hematoxylin (Grübler) aqueous; picrocarmine aqueous; Delafield's hematoxylin; safranin, both aqueous and alcoholic; Flemming triple stain and alcoholic Heidenhain's hematoxylin. The ferric chloride method used by Chalkley and Daniel (1933) proved very unsatisfactory as compared with other stains and consequently was used but little in this work. Heidenhain's hematoxylin was used in both long and short methods. Differentiation was done with aqueous iron alum and in other cases with a saturated aqueous solution of picric acid and ammonia following the method recommended by Kidder (1934). Flemming triple stain was used with sectioned material in a few instances but in such cases only the safranin stained. Concentrated aqueous safranin was used regressively and gave a very brilliant red color. Alcoholic safranin (Lee) in anilin water produced very good results in sections showing dividing chromatin.

With the Feulgen nuclear reaction, contrary to our experience with *Amœba dubia* and also to results generally obtained with rhizopods, a positive reaction was obtained. We agree in this respect with Chalkley's (1936) findings. In some cases, counterstains such as eosin were used after Heidenhain's hematoxylin.

The best results were obtained using alcoholic Heidenhain's hematoxylin which was prepared in the following manner. A ripened stock solution (10 per cent in absolute alcohol) was used to make a 0.5 per cent solution in 70 per cent alcohol. As a mordant with this stain, 4

grams of iron alum in 100 cc. of 70 per cent alcohol were used. It was found that much better results could be obtained when the staining was done at a temperature of 40° C. for about 15 minutes, i.e., mordant for 15 minutes and stain for 15 minutes. For differentiation the mordant solution was diluted twice. Differentiation was accomplished under a binocular dissecting microscope or the low power of the compound microscope. After differentiation, slides were transferred to 50 per cent, 30 per cent alcohol and then washed thoroughly in running tap water for 30 minutes. Dobell (1914*b*) found that this procedure gave excellent results in similar work.

Nuclear Structure of Vegetative Amœba

Leidy (1879) describes the nucleus in the living animal as being "colorless, homogeneous, indistinctly and finely granular, or more coarsely, uniformly, and distinctly granular. I did not at any time distinguish a distinct membranous wall to the nucleus; and a distinct nucleolus, if present, escaped my notice." Calkins (1898) found in sectioned *Amœba proteus* that the nucleus consisted of a more deeply staining substance—"chromatin in the form of granules distributed throughout the nucleus; the other substance has the form of a disk lying in the center of the nucleus." According to Schaeffer (1916) the nucleus in the living *Amœba proteus* is "typically discoid, sides of the disk flat, slightly convex or slightly concave; sometimes dented or invaginated, especially in old individuals; conspicuous, except immediately after division; size of discoid nucleus, average diameter 46 μ and average thickness, 15 μ. Chromatin in several thousand masses arranged in one (?) layer under the nuclear membrane." In a later paper (1926) the same author classes the nuclei of *Amœba proteus* (*Chaos diffluens*) and *Amœba dubia* (*Polychaos dubia*) under the same general type and states that in this type the chromatin occurs in the living 'resting nucleus' "in a layer of small grains of uniform size at a greater or less distance from the nuclear membrane. . . . In addition to these masses of chromatin there are found, after fixing and staining, other masses of stainable matter, usually irregularly placed, nearer the center of the nucleus, and in some species 'clouds' or concentric rings of fine dust-like stainable particles, whose chromatin constitution has not yet been fully established." Doflein (1918) who, in our opinion, undoubtedly worked with *Amœba proteus* gives a description of the vegetative nucleus which generally conforms with those given by Calkins and Schaeffer. The Binnenkörper, he states, is not sharply marked off from the outer nuclear region although he figures it as a distinct body. In gen-

eral its shape conforms to that of the entire nucleus. In Taylor's (1923) figures of the resting nucleus of *Amœba proteus* peripheral granules and karyosome are shown and her description is essentially similar to that of Doflein. Bělař (1926) states that the so-called Binnenkörper of *Amœba proteus* and related forms is a fixation artefact. Chalkley and Daniel (1933) working with *Amœba proteus* state that "the chromatin, . . . in the resting nucleus is distributed in granules or 'blocks' immediately beneath the nuclear membrane." Further description of the vegetative or resting nucleus is lacking although mention is made of the karyosome during the initial stages of mitosis. Later, Chalkley (1936) states "the granules of chromatin (sic) that lie in interkinesis just beneath the nuclear membrane play no part in the formation of the chromatin of the equatorial plate, . . . the chromatin granules of the plate arise entirely from the karyosome."

The nuclear membrane of the resting nucleus of *Amœba proteus* is clearly defined in both the living and the stained preparations. Immediately under the nuclear membrane is a layer of deeply staining granules as described by various other authors. The size of these granules varies from 1 to 2 μ in diameter. From the study of total mounts alone a very inadequate idea is had of the nature of the remaining part of the nucleus. In stained sections the central portion of the nucleus may be accurately studied. Such preparations (Figs. 19–26) show the central portion of the nucleus to be composed of a lightly staining fairly homogeneous mass of finely granular material. To ascertain whether or not chromatin was present in this part of the nucleus the Feulgen nuclear reaction was used. By this method (Figs. 28 and 29) both the peripheral granules and the central portion stained, the former being somewhat more intense in its reaction. According to Chalkley (1936) the peripheral granules "contain at best merely traces of nucleic acid." Similar results were obtained by the use of Heidenhain's hematoxylin after various kinds of fixation. Depending on the fixative used it was found, as Bělař (1926) has pointed out, that the presence of a clearly marked "karyosome" is evidence of a fixation artefact (Figs. 27, 28 and 29). Following the suggestion of Calkins (1933), the term endosome will be used in this work in reference to the central portion of the nucleus of *Amœba proteus*.

The Process of Fission as Seen in the Living Amœba

The formation of division spheres and the subsequent changes in the form of pseudopodia are very similar to those previously described by the writers for *Amœba dubia* (1935). See Plates I and II. Doflein (1918) figured the division sphere of *Amœba proteus* and Levy (1924)

presented outline sketches showing the various changes in the division sphere during fission in this species. The most accurate drawings showing this process are those of Chalkley and Daniel (1933).

The "hyaline area" indicating the position of the nucleus during early division phases mentioned by Chalkley and Daniel could usually be seen under the binocular dissecting microscope. The present authors found, as in *Amœba dubia,* cytoplasmic currents and general internal activity occur, although from external view the animal seems to be completely inactive. Observations on the living division spheres under the highest magnification possible did not reveal the dividing nucleus. Various procedures were used in preparing the living division spheres for study. In none of the preparations, including those made by the agar method kindly suggested by Dr. Chalkley, could any of the early stages in nuclear division be seen. At the time of cytoplasmic division, nuclei could, however, be readily observed.

As shown in Plates I and II the cytoplasmic division may follow one of two types both of which also may occur in *Amœba dubia.* The first type of division is shown in Plate I. Here in Figs. 1–5 are shown slight progressive changes in a living division sphere prior to elongation. A slight elongation can be noticed in Fig. 5. In Fig. 6 projections indicate the beginning of formation of the daughter amœbæ. These projections grow, pushing out in opposite directions and the remaining portion of the division sphere becomes drawn out into a cylindrical form as in Fig. 7. The connecting cylindrical part gradually becomes thinner and the daughter cells have numerous short, blunt pseudopodia. As in *Amœba dubia,* cytoplasmic currents can be observed to flow, first in one direction and then in the other in this connecting strand. The pulling out process proceeds rapidly (Figs. 8–11) until, just before the connecting strand breaks, all motion of cytoplasm within it ceases. Each broken end snaps back toward its respective daughter amœba becoming broader and thicker as it is withdrawn. It is interesting to note that this type of division although most frequently met with in *Amœba dubia* occurred less frequently in *Amœba proteus* than the second type.

In the second type of cytoplasmic division no long connecting strand can ever be seen. The stages preceding either type of division are similar up to the point (Fig. 6) where indications of the forming daughter amœbæ become obvious. The two daughter amœbæ flow out very rapidly forming very large coarse pseudopodia (Figs. 12 and 13). The connection between them as shown in Fig. 14 is very short as compared with that in Fig. 11. From this point separation occurs within a few seconds leaving the daughter amœbæ lying close together.

In some instances it is difficult to detect the complete division until after fixation (Fig. 18).

Our results agree closely with those of Chalkley and Daniel (1933) in respect to the duration of the process of fission in *Amœba proteus*. In general it has been found that the correlations between pseudopodial width and stage of nuclear division as described by the above-mentioned writers are roughly correct. In our experience, however, wide divergences may exist between pseudopodial configuration and the stage of nuclear division. Division spheres which had already begun to elongate showed, upon fixation and staining, early stages of division. The pseudopodia are coarser in the later stages of fission. In this work, however, it has been found impossible to isolate any desired stage of nuclear division with the 97 per cent accuracy claimed by Chalkley and Daniel (1933).

The Prophase

The resting nucleus of *Amœba proteus* which is entering on the prophase becomes considerably swollen so that when observed in surface view, although still discoid in shape, the slight, biconcave depressions have now pushed out. The nuclear membrane shows clearly, being somewhat thinner than in the vegetative condition. The peripheral granules still stain fairly strongly and have now decreased in number. They are much less uniform in size and more widely separated. At the same time there is forming, in the central portion of the nucleus (endosome) a mass of numerous small, deeply-staining, rod-like granules which are all definitely less than 0.5 μ in length (Figs. 43, 44, 45 and 60). These granules have a plate-like arrangement.

Spindle fibers are lacking at this stage. According to Chalkley and Daniel "the chromatin has left the membrane and is evidencing a tendency to aggregate in a zone . . . the chromatin appears completely

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EXPLANATION OF PLATE I

Photomicrographs. All figures were photographed using a Leitz compound microscope and Leitz apochromatic lenses. Number 3 objective and 15 × oculars were used in all cases. All figures were made from living amœbæ in process of division. Magnification approximately 90 diameters.

Figs. 1–5. *Amœba proteus* Pallas (Leidy). Showing successive stages in division spheres prior to elongation. Compare with Figs. 17 and 18.

Fig. 6. Division sphere showing beginning formation of daughter amœbæ.

Fig. 7. Slightly later.

Figs. 8–11. Showing cytoplasmic bridge connecting daughter amœbæ in successive stages. Type I division. See text.

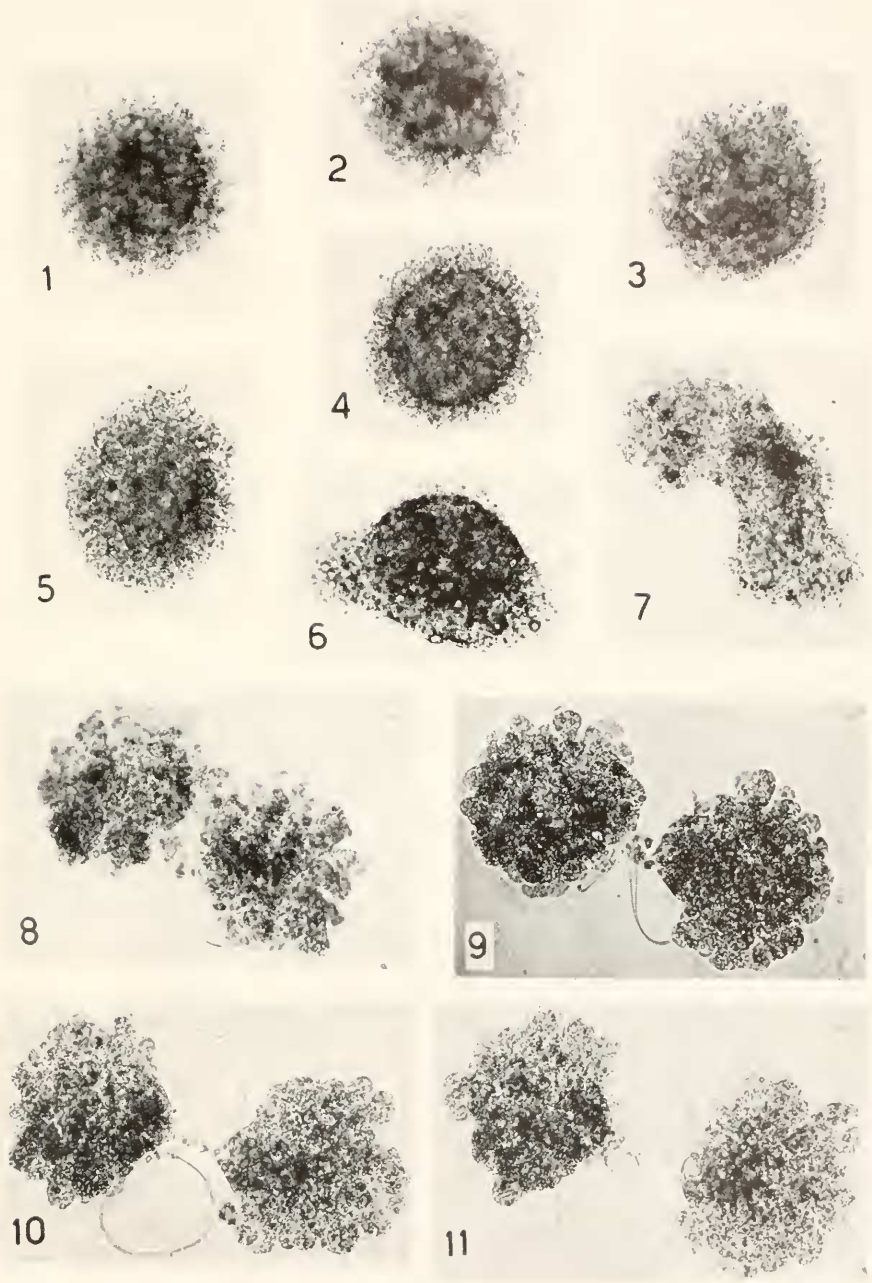

PLATE I

withdrawn from the membrane . . . tending to form an irregular ring." Later (1936) Chalkley retracts this statement saying that the peripheral granules play no part in the formation of the equatorial plate. In our preparations no evidence can be found that the peripheral granules leave their position close to the membrane and migrate to a central portion of the nucleus. On the contrary, they gradually fade out of the picture and take no part in the formation of the plate. In preparations of this stage very definite evidence is present to show that the granules which are to form the plate are at no time arranged in a ring but are in the form of a disk.

The Metaphase

Beginning with the definitely formed metaphase plate as shown in Figs. 30–35, 46 and 61 the following condition is found. The nuclear membrane is still present. This is especially clear from our sections. Fibers are now seen extending from the plate to the nuclear membrane. At this stage, due to the shape of the nucleus and the delicate character of the membrane at the polar regions of the spindle, multipolar appearances have been observed. This effect is, we believe, due solely to fixation artefacts. The plate is composed of a very large number of individual, deeply-staining small chromatin granules. It is approximately circular in outline, the diameter being about 25 μ. The granules now are $\pm$ 0.3 μ in diameter and are quite uniform in size. In a few preparations, especially in sectioned material, larger achromatic granules may be observed. These lie outside of the plate among the spindle fibers. It is believed that these bodies are remnants of the former peripheral granules which have not yet disintegrated and that they take no part in the mitotic process (Figs. 35 and 46).

The dividing chromatin of the metaphase plate in *Amœba proteus*

EXPLANATION OF PLATE II

Photomicrographs. Figures 12–15 from living amœbæ in process of division. Magnification approximately 90 diameters.

Figs. 12–15. *Amœba proteus* Pallas (Leidy). Successive views of final stages in fission. Type II. Compare with Plate I.

Fig. 16. Total mount. Division sphere in prophase. Heidenhain's hematoxylin. × 70.

Fig. 17. Total mount. Division sphere in late anaphase. Safranin. × 400.

Fig. 18. Total mount. Cytoplasmic division, Type II. Note peripheral position of nuclei. Heidenhain's hematoxylin. × 400.

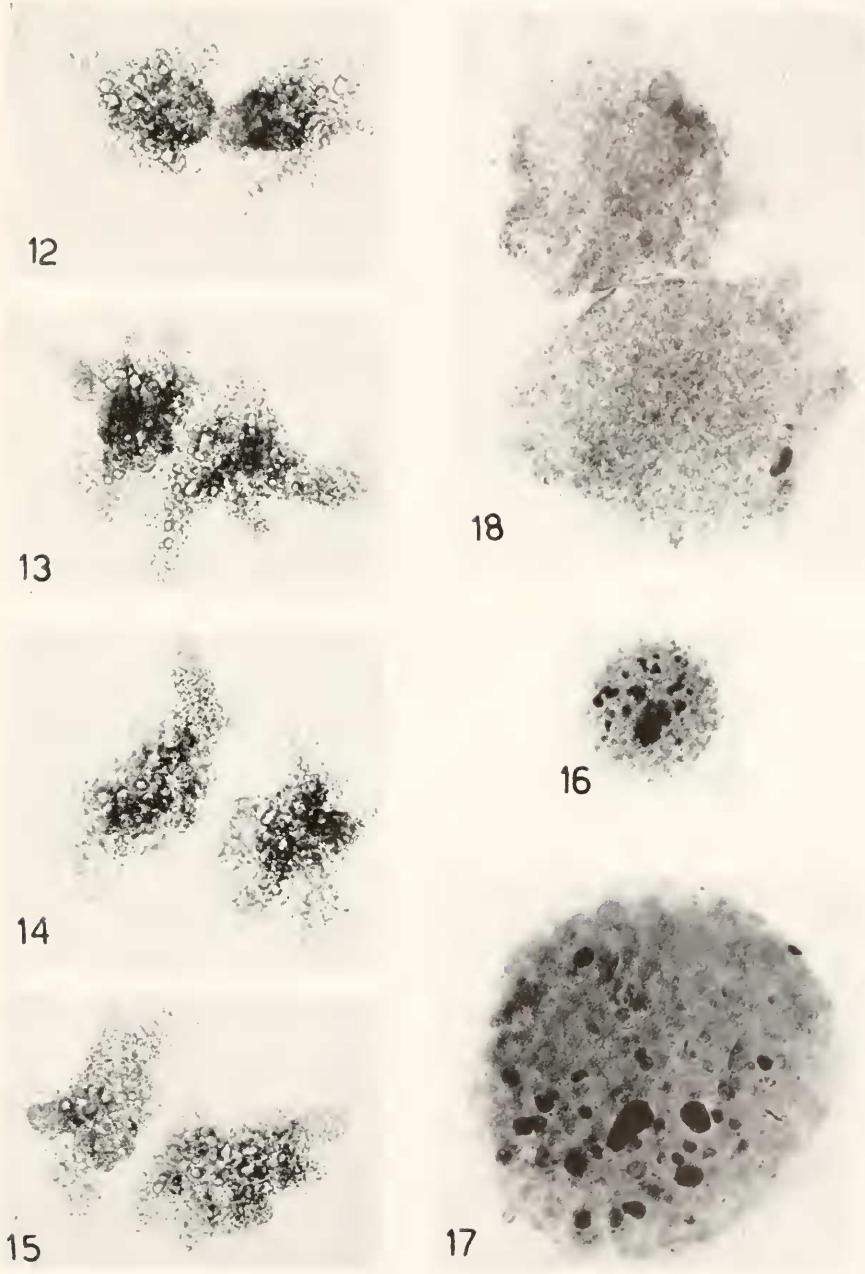

PLATE II

stains heavily with all basic dyes used and gives a strongly positive Feulgen nuclear reaction. This is in sharp contrast with the condition in *Amœba dubia* in which no sharply defined metaphase plate was observed.

The Anaphase

In the early anaphase the former metaphase plate has divided to form two distinct plates (Figs. 36, 37, 47, 48 and 62). The nuclear membrane, although delicate, is still present and may be traced completely around the figure, especially in sectioned material (Figs. 36 and 62). The spindle fibers extending between the two separating plates can be clearly seen at this stage. They are extremely numerous. The polar fibers now definitely terminate at a point, giving a bipolar spindle. The chromatin granules comprising the plates are similar in size and staining capacity to those of the preceding stage. The larger achromatic granules now occur less frequently but a few may be observed in sectioned material (Figs. 36, 37, 47 and 48).

As the anaphase progresses the daughter plates become more widely separated (Figs. 38, 39, 40, 49 and 50). The nuclear membrane is still present. It is now very delicate and cannot be seen in all total mounts. In sections, however, its presence may still be detected. In

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EXPLANATION OF PLATE III

Photomicrographs. All magnifications × 1,200. All figures from sectioned amœbæ. Sections 6 μ thick. All fixed in Flemming except Figs. 27–29 which were fixed in Schaudinn's. All stained with Heidenhain's hematoxylin except Figs. 28 and 29 (Feulgen) and 30–34 (safranin).

Figs. 19–21. Vegetative nucleus serially sectioned through surface. Note deeply stained peripheral granules and in Fig. 20 endosome showing throughout.

Figs. 22–26. Vegetative nucleus serially sectioned at right angles to long axis.

Fig. 27. Vegetative nucleus showing endosome as fixation artefact.

Figs. 28 and 29. Vegetative nucleus. Feulgen. In both the peripheral granules stain deeply. Fixation as in Fig. 27.

Figs. 30–34. Serial section of metaphase plate. Note spindle fibers and nuclear membrane. Compare with Fig. 46.

Fig. 35. Early metaphase plate. Note achromatic granules and nuclear membrane.

Figs. 36 and 37. Successive views, early anaphase. Note nuclear membrane, divided plate, bipolar spindle and achromatic granules. Compare with Figs 47 and 48.

Figs. 38–40. Serial sections through mid-anaphase. Note nuclear membrane especially at polar regions, spindle fibers and achromatic granules. Compare with Figs. 49 and 50.

Figs. 41 and 42. Telophase, side and surface views from same amœba. Note "parachute" in Fig. 41 and convex type of plate. No achromatic granules present. In Fig. 42 note nuclear membrane and fine granulation. Compare with Fig. 54.

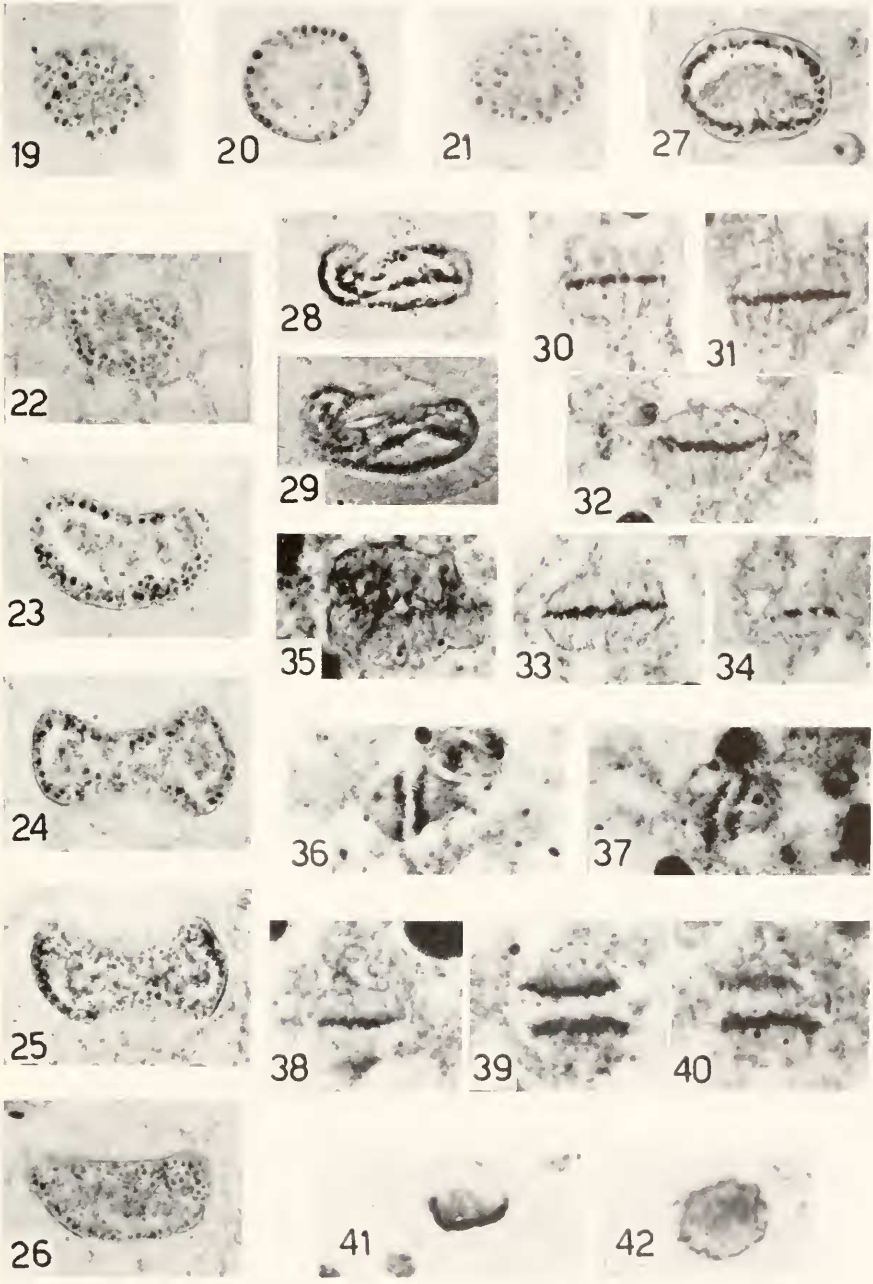

PLATE III

mid-anaphase the spindle fibers are numerous and clear as are also the polar fibers. An excellent idea of the nature of the daughter plates in this stage may be had from a three-quarter view as shown in Figs. 51 and 63. The size and disposition of the chromatin granules in the plates are clearly shown.

The plates move farther apart and their planes still remain in most cases parallel. Each gradually becomes saucer-shaped with the convex surfaces facing each other (Fig. 52). The polar spindle fibers are still present but the fibers between the plates have practically disappeared. At this stage a few achromatic granules may still be seen in some preparations. In a similar stage in *Amœba dubia* the achromatic granules are relatively numerous.

The nuclear membrane so clearly visible in *Amœba dubia* at this stage becomes difficult to follow in *Amœba proteus*. In our preparations the nuclear membrane is present, surrounding the polar regions

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EXPLANATION OF PLATE IV

Photomicrographs. All magnifications × 1,200. All figures from total mounts except Figs. 55–59 which are from sections, 6 μ thick. Figures 43–54 fixed in Carnoy-Lebrun. With the exception of Fig. 58 all remaining figures from amœbæ fixed in Flemming. All stained with Heidenhain's hematoxylin.

Figs. 43–45. Nuclei in prophase. Note peripheral granules and cloud-like forming plate. Compare with Fig. 60.

Fig. 46. Metaphase plate. Note achromatic granules. Compare with Figs. 30–35 and Fig. 61.

Figs. 47 and 48. Early anaphase. Two views of same figure at different focus. Note separating plates, spindle fibers and achromatic granules. Compare with Figs. 36, 37 and 62.

Figs. 49 and 50. Mid-anaphase. Two views of same figure at different focus. Note spindle fibers. Compare with Figs. 38–40.

Fig. 51. Mid-anaphase. Three-quarter view showing granulation of plates. Compare with Fig. 63.

Fig. 52. Late anaphase. Note curving of plates, polar fibers and achromatic granules. Compare with Fig. 17.

Fig. 53. Early telophase. Note greater curvature of plates and achromatic granules. Compare with Fig. 64.

Fig. 54. Later telophase showing one nucleus in side and one in surface view. Note character of granules and forming "parachute." Compare with slightly later condition in Figs. 41, 42 and 65.

Figs. 55–59. Showing reconstructing nuclei. Figure 55 one-half hour after division. Note small size of peripheral granules and vacuolated endosome. Figure 57. Two hours after division. Note typical nuclear shape. Peripheral granules increased in size. More granular.

Fig. 58. Three hours after division. Note increase in size of peripheral granules and those of the endosome. Compare with Fig. 68. Sectioned in surface view.

Fig. 59. Five hours after division. Note similarity to vegetative nucleus.

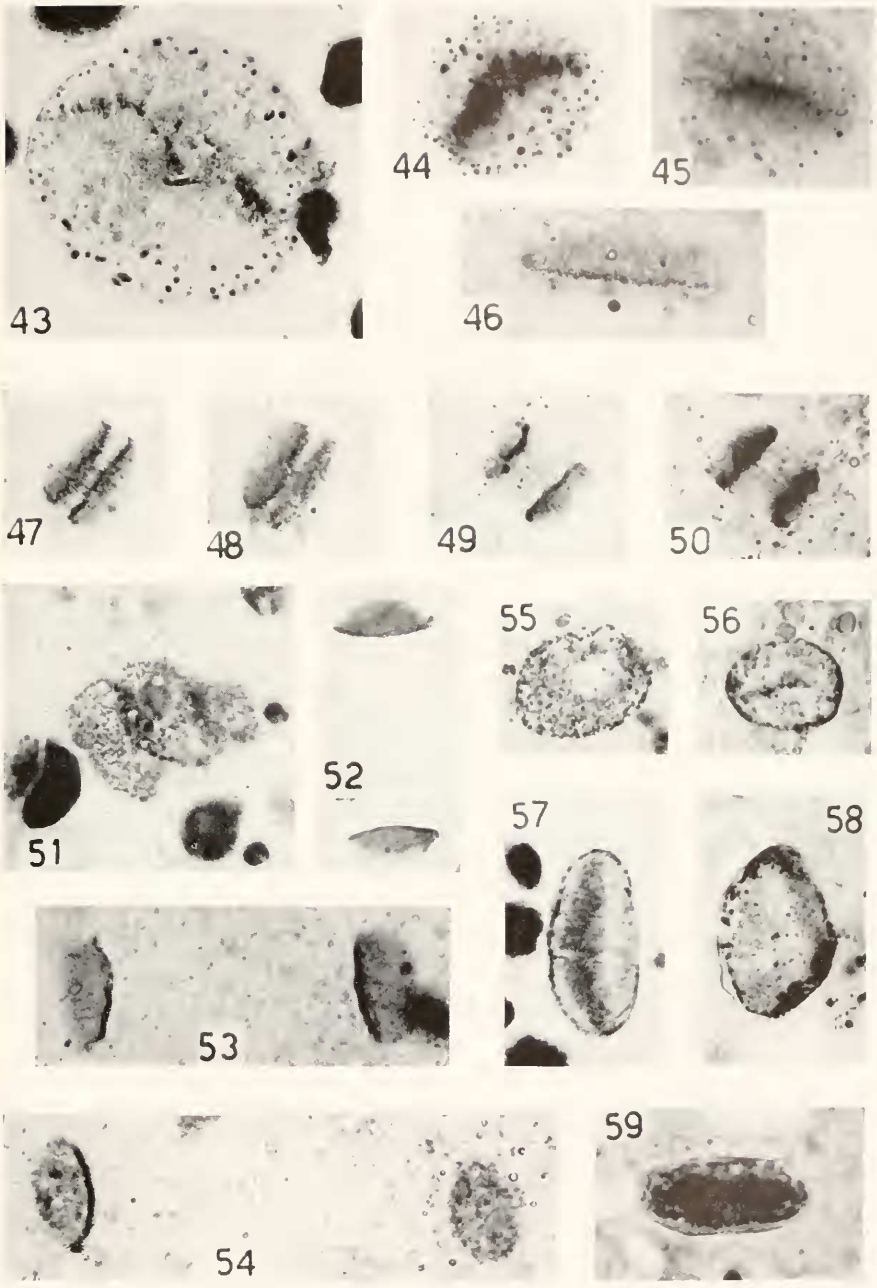


PLATE IV

of the separating plates. It is difficult to make out with certainty on the inner surfaces of the plates. Some evidence of invagination or pinching off of the membrane in the equatorial zone of the spindle has been obtained. We believe that the membrane does not break down but divides in this manner just after mid-anaphase and thus comes to encircle each daughter nucleus.

The Telophase

The telophase stage is characterized by the further migration of the daughter plates (nuclei). At this time the division sphere has definitely elongated and the daughter plates (nuclei) are found near its poles. An early telophase is shown in Figs. 53 and 64. The daughter plates are now thicker, somewhat more convexly curved, and consequently have a shorter diameter. Measurements show the diameter now to be about 11 to 12 μ. The nuclear membrane appears much more distinct. The polar spindle fibers form a conical cap on the concave surface of the plate. No sign of interzonal fibers can be seen. At the same stage in the division of *Amœba dubia* such fibers are characteristically present. The disappearance of the achromatic granules is practically complete at this time.

Later stages of the telophase are associated with cytoplasmic division of the animal. Typical later telophases are shown in Figs. 18, 41, 42, 54 and 65. The nucleus may now best be described by calling it a "parachute." The polar spindle fibers represent the shrouds of the parachute and the plate, the dome. Such structure is most apparent in sections one of which is shown in Fig. 42. In surface view (Figs. 42 and 54) one can observe that the granules originating from the plate

Explanation of Plate V

All drawings made with camera lucida. × 1,700.

Fig. 60. Same as Fig. 43. Nucleus in prophase showing peripheral granules clearly defined and forming plate.

Fig. 61. Similar to Fig. 33. Median section through metaphase nucleus showing dividing plates, spindle fibers and nuclear membrane.

Fig. 62. Same as Fig. 36. Early anaphase showing divided plates, bipolar spindle, spindle fibers and nuclear membrane.

Fig. 63. Same as Fig. 51. Mid-anaphase, three-quarter view.

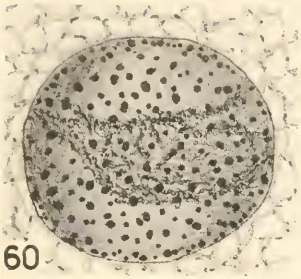
Fig. 64. Similar to Fig. 53. Showing early telophase condition. Note characteristic saucer shape of plate and achromatic granules.

Fig. 65. Late telophase nucleus. "Parachute" stage.

Fig. 66. Reconstructing nucleus ten minutes after division. Note uniform distribution of granules and delicate nuclear membrane.

Fig. 67. Reconstructing nucleus one and a half hours after division.

Fig. 68. Reconstructing nucleus three hours after division. Note presence of peripheral granules.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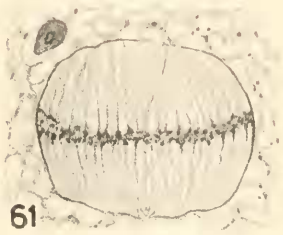
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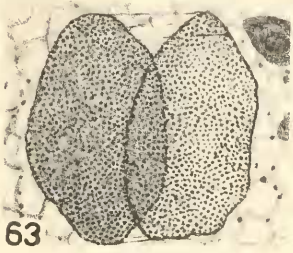
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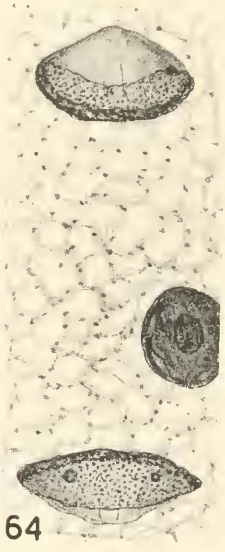
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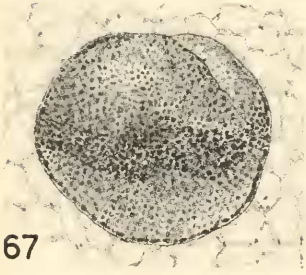
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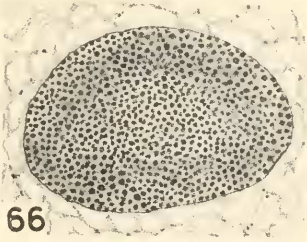
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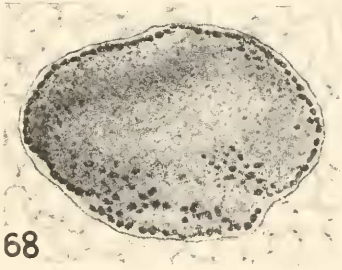
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PLATE V

are extremely fine and numerous. They do not stain so intensely. The diameter of the granules is unchanged. The diameter of the nucleus in surface view varies from 12 to 15 μ whereas the measurement of the short axis of the nucleus is approximately 4 to 5 μ At this stage but one type of staining granule is present and the nucleus is obviously much smaller than in the adult vegetative condition.

Reconstructing Nucleus

Although at the time of cytoplasmic division the nucleus has a definite membrane, it can by no means be considered completely reconstituted as Chalkley and Daniel (1933) state. We have studied the reconstituting nucleus both in total mounts and in sections from the time of division to over 5 hours thereafter. At the moment of division there is no evidence of the characteristic biconcave, discoidal shape (Fig. 18) but it resembles a thin saucer. Ten minutes after division it becomes slightly biconcave and measures approximately 20 μ in the long diameter and 6 μ in the short diameter. The former plate still occupies its central position in the nucleus. In surface view it is seen to be composed of numerous, extremely small (less than 0.3 μ in diameter) granules which stain less intensely than those of the telophase. During the interval between cytoplasmic separation and ten minutes after division, peripheral granules first make their appearance. These, in the beginning, are indistinguishable in size and staining capacity from the granules of the plate.

From 10 to 30 minutes after division the nucleus becomes slightly larger being approximately 24 μ in the long axis at 30 minutes. The peripheral granules also increase slightly in size now measuring from 0.5 μ to 0.7 μ in diameter. They appear somewhat irregularly placed and seem to have fibrillar connections. The central region which marks the former position of the plate becomes slightly granular, vacuolated and stains more faintly (Figs. 55 and 66).

At the end of one hour after division the nucleus is approaching normal vegetative size, measuring through the long axis approximately 30 μ and through the short axis 13 μ. The final definitive shape of the vegetative nucleus has been nearly if not entirely assumed at this time (Figs. 56 and 67). The peripheral granules are slightly larger measuring about 8 μ in diameter. The central portion of the nucleus, the endosome, is now vacuolated, has a reticular appearance and stains more heavily. It is definitely granular and extends throughout the entire nucleus.

Other than a slight increase in size, little change can be noted in the two-hour reconstructing nucleus (Fig. 57). Three hours after division both the peripheral and some of the central granules have increased in size (Fig. 68). The picture is not essentially different four hours after division. The peripheral granules, however, show a perceptible increase in size, being 0.9 μ in diameter. The central mass (endosome) is definitely granular and extends throughout the nucleus. It stains evenly and fairly heavily in sectioned material.

About five hours after division (Fig. 59) at room temperature (in this case 30° C.) it is difficult to distinguish the reconstituting nucleus from the vegetative condition.

Discussion

From the preceding account of mitosis in *Amœba proteus* it is obvious that the process differs markedly from that in *Amœba dubia.* It is also to a certain extent at variance with the accounts given by other workers with the same species. Descriptions of the vegetative nucleus of *Amœba proteus* by former workers agree in general that a nuclear membrane is present and that a layer of peripheral granules underlies this membrane. In regard to the structure and function of the central portion of the nucleus or endosome considerable diversity of opinion exists. Thus Taylor (1923) describes an endosomal body which is clearly marked off from the outer region of the nucleus. Doflein (1918), although he does not specifically state a similar condition, has figured it as such. Our observations have led us to agree with Bělař (1926) that such a sharply marked-off endosome is evidence of faulty fixation and our study of the structure of the vegetative nucleus of *Amœba proteus* convinces us that the account given by Calkins (1898) is essentially correct. We are unable to confirm Schaeffer's statement (1926) that the vegetative nuclei of *Amœba proteus* and *Amœba dubia* belong in the same category. In *Amœba dubia* no endosome as such could be seen but the staining granules were evenly distributed throughout the nucleus. We believe that the uniformly distributed mass of relatively large granules in the *Amœba dubia* nucleus are homologous to the peripheral granules of the *Amœba proteus* nucleus. Both disappear during mitosis and both are reformed "de novo" from the dividing chromatin.

Doflein (1918), Bělař (1926) and Chalkley and Daniel (1933) all hold that the peripheral granules are the dividing chromatin and that the endosome supplies no chromatic elements. It is noteworthy that Chalkley in his recent brief account (1936) completely reverses his opin-

ion in this regard and states that "these granules give rise to, or contribute to, the formation of the spindle fibers and the pole caps." Our work shows that the peripheral granules begin to disintegrate during the prophase and do not migrate to the central portion of the nucleus where the metaphase plate is forming. The metaphase plate originates from the endosome.

The multipolar metaphase figures shown by Carter (1912) and Doflein (1918) are, as can readily be seen from our photographs and figures, solely due to artefacts of fixation. It is noteworthy that such figures are shown from sections. We have found that the so-called "multipolarity" is accentuated in sections through the outer portion.

Chalkley and Daniel[1] (1933) claim that the nuclear membrane disappears at metaphase. Although Carter (1912) figures a nuclear membrane surrounding the metaphase figure, Doflein (1918) questions this and states that the nuclear membrane is not present at this stage. On the contrary, we have found the nuclear membrane clearly defined as late as mid-anaphase and also present in the early telophase. Indications of its presence in the intervening short period (at most five minutes) may also be seen. We therefore believe that the nuclear membrane does not break down during mitosis in *Amœba proteus*.

Our observations on the reconstructing nucleus in *Amœba proteus* have led to a better understanding of the origin of the peripheral granules. These begin to reappear just after cytoplasmic division and gradually grow in size with the growth of the nucleus. The genesis of these granules is decidedly similar in *Amœba dubia* and we are forced inescapably to the conclusion that they originate from the plate material, i.e., they are endosomal in origin.

Binary fission in *Amœba proteus* and *Amœba dubia* represents, we believe, the method of reproduction. The conception of Doflein that multiple division is a regular occurrence in *Amœba proteus* is, in our opinion, erroneus. The critical review by Johnson (1930) concerning

[1] In a more complete paper appearing in December, 1936, shortly before this paper went to press, Chalkley stresses again the absence of the nuclear membrane during metaphase and anaphase in the division of *Amœba proteus*. Evidence is presented by us in this paper to show that the nuclear membrane is present during metaphase and early anaphase. We are in accord with Chalkley in finding that the dividing chromatin in *Amœba proteus* originates in the karyosome (endosome), but it is difficult to follow his reasoning when he states that the polar caps, the spindle fibers and possibly the "new nuclear membrane" are formed in part from the peripheral granules. A study of his paper fails to reveal evidence for such a belief. It does seem entirely probable, however, that the so-called peripheral chromatin is derived from the karyosome. The fact that it gives the Feulgen reaction for a short period during reconstruction while it is migrating from the karyosome to the periphery would clearly indicate that such is the case.

diverse theories of reproduction in the large free-living species of *Amœba* and the recent work of Halsey (1936) serve to confirm this belief.

Summary

1. In *Amœba proteus* the vegetative nucleus is a discoid, biconcave structure with a nuclear membrane, a layer of peripheral granules and a central endosomal mass.

2. During prophase the peripheral granules begin to disintegrate and the dividing chromatin originates from the endosome.

3. In metaphase the plate is fully formed and consists of numerous, small, deeply-staining chromatin granules. Spindle fibers, both interzonal and polar, are first apparent at this time. The interzonal fibers disappear at late anaphase. The polar fibers persist throughout the process.

4. The granules of the metaphase plate divide to form the two plates of the anaphase which continue to separate, becoming condensed and curved until the telophase stage.

5. The nuclear membrane is clearly present up to mid-anaphase and again at telophase. There is evidence that it persists throughout the entire process of mitosis.

6. The nucleus is fully reconstituted about five hours after cytoplasmic division. The peripheral granules are formed from the plate mass and appear a few minutes after division.

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